INVESTIGATING ICE SLURRY'S PERCEIVED MECHANICAL ABRASIVE QUALITY TO INCREASE PATHOGEN REDUCTION ON POULTRY DURING IMMERSION CHILLING

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INVESTIGATING ICE SLURRY'S PERCEIVED MECHANICAL ABRASIVE QUALITY TO INCREASE PATHOGEN REDUCTION ON POULTRY DURING IMMERSION CHILLING

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To all the people who still eat poultry and all the people who make Georgia
#POULTRYPROUD

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It takes a village to raise a child. My village is made of residents who I've collected along the years. I thank my parents for the engrained work ethic I've developed by watching you in times of struggle. I thank my Lao Niang, my petite fire-cracker grandmama, for teaching me Mandarin and showering me with so much love.

Dill-Pickle, #WeMadeIt.

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LIST OF SYMBOLS AND ABBREVIATIONS

BPW	Buffered Peptone Water
STR	Nalidixic-acid resistant *Salmonella* Typhimurium
USDA	United States Department of Agriculture
FSIS	USDA Food Safety and Inspection Service
WOG	Whole carcasses without the giblets
PAA	Peracetic Acid Antimicrobial
ppm	Parts-Per-Million
PBS	Phosphate Buffered Saline
CFU	Colony-Forming Unit
TSB	Tryptic Soy Broth
EHS	Georgia Tech Environmental Health and Safety
Δ_S	Slurry STR reduction from baseline
Δ_W	Water STR reduction from baseline
$\Delta_s - \Delta_w$	Slurry added reduction of STR over water
T_i	Initial carcass temperature
T_f	Post-chilling carcass temperature
ΔT	Carcass temperature reduction from chilling
M_i	Initial carcass mass
M_f	Post-chilling carcass mass
ΔM	Carcass mass difference after chilling

SUMMARY

The poultry industry is an integral part of Georgia's economy, accounting for more than half of the state's agricultural output. In Georgia, more than 20 million pounds of broiler meat are produced daily. Poultry processing entails many phases, and this thesis focuses upon the immersion chilling step. The chilling phase is critical to reducing pathogen presence and ensuring meat product shelf-life. Immersion chilling consumes intense amounts of water and energy resources, and the industry is trying to discover more efficient approaches for processing.

This thesis is based upon a multi-year project investigating ice slurry as an alternative chilling medium for the poultry industry. Ice slurry is composed of small characteristic length ice particles and a salt-brine solution that acts as a freezing point depressant. The salt is an important component in maintaining the ice slurry in a homogenous state (i.e., reduce ice agglomeration and media separation). Ice slurry is hypothesized to provide a disruptive scrubbing/abrasive phenomenon resulting in greater pathogen reduction compared to tradition chilled water medium.

Pathogen reduction experiments were conducted to determine the *Salmonella* pathogen reduction capability of chilled water and ice slurry on whole carcasses and wing-parts. Pathogen reduction experiments combined experimental factors of: peracetic acid (PAA) antimicrobial concentration, media salinity, time of immersion chilling, and air agitation levels. Treatment combinations were compared to discover the optimum

relationship between factors resulting in the best reduction from STR concentrations pre- and post-chilling.

The project also investigated the salt-uptake tendencies of whole carcasses during immersion chilling. Whole carcasses, without the giblets (WOGs) were chilled by either air chill, chilled water, or 4.5% salinity chilled water. Post-chilling, three sample types were collected per each carcass (breast skin, white meat, and dark meat). Results initially addressed salt-uptake concerns when ice slurry medium is used for immersion chilling. Initial findings indicated that salt concentrations increased in the skin, yet did not affect white or dark meat. The skin acts as a barrier that prevents salt penetration into the white and dark meat, furthering the consideration of ice slurry as a poultry chilling medium.

CHAPTER 1. THESIS OVERVIEW AND LITERATURE REVIEW

1.1 Thesis Overview

Ice slurry, a two-phase cooling medium was investigated in four independent poultry immersion chilling studies. Each study is presented within respective chapters (i.e., Chapters 2-5). Results and discussion are included in each chapter, while the conclusion is a combination of all independent studies and how they interplay.

1.2 Literature Review

This section references published information on topics covered in this thesis. Background information is provided about the poultry industry, concerns and hurdles associated with food safety, addressing poultry concerns, and how ice slurry may address some of these challenges.

1.2.1 Poultry Processing and Steps

Poultry primary processing begins with market-weight live broiler chickens (from four to eight weeks of age**)** and concludes in consumer product. United States' processing plants follow a general processing flow, detailed in **Error! Reference source not found.**(Barbut, 2015). Upon arrival at the processing plant broilers are placed upside down in shackles from where the slaughter step begins (Smith, 2014). The shackle line feeds the birds through an electric water bath, stunning them unconscious before a blade

severs the neck. After draining the blood, the now dead carcasses move through a series of scalder baths. The scalding bath (at a minimum 47°C), loosens the feather from the follicles and dislodges attached debris. The shackles then feed through a picking tunnel which is a machine with attached rotating rubber picker fingers that apply pressure to remove feathers and any debris from the carcass. The heads are then removed before the evisceration stage.

Prior to the evisceration stage, carcasses are transferred to a new shackle line. The oil gland and feet are removed, and the abdominal and thoracic viscera are extracted via an eviscerator. The carcasses viscera are displayed for a UDSA mandated post-mortem inspection. This inspection checks for disease and defects of the carcasses. After passing inspection, the carcasses, now considered whole birds without the giblets (WOG) proceed to the chilling process. After chilling, WOGs are either packaged whole or moved to secondary/ further processing.

The Poultry Processing Line					
1	Unloading of Live Birds	2	Hung by Feet on Shackles	3	Electrical Stunning and Bleeding
4	Water Scalding	5	De-feathering	6	Oil Gland and Feet Removal
7	Evisceration	8	USDA Mandatory Inspection	9	Immersion Chilling
10	Weighing and Grading	11	Portioning and Packaging	12	Transport of Packaged Product

Figure 1.2.1 Poultry primary processing general model of step sequence.

1.2.2 Chilling Focus

Immersion chilling is a vital step in ensuring food safety (Buhr RJ et al., 2005). Poultry carcasses are chilled to suppress carcass pathogen growth presence (James, 2005)

while antimicrobial chemicals reduce pathogen concentrations. Prior to chilling carcass temperatures are approximately 40^0 C, The United States Department of Agriculture (USDA) Food Safety and Inspection Service (FSIS) used to require carcasses to be cooled and maintained to ≤ 4°C as soon as possible (Barbut, 2015). The temperatures of ≤ 4°C must be confirmed in the internal breast meat as it is the slowest cooling location within the carcass. The chilled temperatures ensure meat product quality and consumer safety (James, 2005). Common chilling methods employed in the poultry industry are air chill, spray chill, and cold water immersion chill. Each chilling method has associated pros and cons detailed in

Figure 1.2.2 (Demirok, 2013; Rodrigues et al., 2014).

METHOD	PRO	CON
Air Chill	Lower cross contamination levels between carcasses	Longer chilling times, less efficient heat transfer
Spray Chill	Lower cross contamination levels between carcasses. Antimicrobial can be applied with spray	Expensive technology and longer chilling intensive
Immersion Chill	Low cost, faster chilling times, antimicrobial can be applied in chiller medium	High energy and water consumption

Figure 1.2.2 Chilling method trade-offs that plants use to consider application utilized.

In the United States, 95% of plants use water immersion chilling (N. A. Cox et al., 2010); Rodrigues, 2015). Immersion chilling is a faster technique that is cost effective. The carcasses' immersion dwell period can range from one to three hours, depending on meat product type. Drag and auger chillers are the main types used in industry, and they are named for the methods used to move carcasses through the chiller (N. Cox et al., 2014). An auger chiller, on-loan from USDA-ARS, was used for WOGs (Chapter 2)

testing. Auger chillers have a screw-auger in the middle that runs the entire length of the chiller (Carciofi & Laurindo, 2007). The motor-controlled auger pushes carcasses to the opposite exit-end of the chiller, where fresh water is added. The fresh water addition exposes the carcasses to cleaner and colder water, hence there is a countercurrent effect.

Carcass cooling rates are dependent upon carcass and chiller properties. Carcass cooling is influenced by its weight, shape, and fat composition. Relevant chiller properties and phenomena include chiller media supply temperature, air agitation flow, media volume, antimicrobial concentration, and carcass load.

Agitation is needed within the chiller for more effective convective heat transfer (Smith, 2014). The standard agitation method comes from compressed air lines feeding into the bottom the chiller. A drawback of air agitation is heating the chiller media (via byproduct heating of air during its compression) (Northcutt, 2008).

1.2.3 Food Safety and Microbiology

The USDA Food Safety Inspection Service (FSIS) protects consumers from food borne illnesses and pathogens through mandated regulations and verification programs. The absence or presence of pathogens in a plant is a good indicator of process management hygiene. Poultry microbiology and food safety pathogens have been studied expansively. A pathogen is considered a fungus, virus, or bacterium that can cause disease in humans. A pathogen can be found on final meat product due to; contamination in the gorwout house, improper feed withdrawal before transport, rupturing the internal viscera during evisceration, unsuitable temperatures during chilling, cross-contamination between WOGs, and inappropriate sanitization methods in the plant. Poultry meat is

most commonly contaminated by bacterial pathogens (James, 2006). Most common problem bacteria in poultry are *Salmonella, Listeria, Campylobacter, Clostridium perfingens,* and *E. coli.* These high-risk bacteria result in huge economic loss every year.

Salmonellosis, caused by *Salmonella*, costs the US over $2600 million annually (Taskila et al., 2012). The USDA *Salmonella* Verification Program from the USDA-FSIS Pathogen Reduction: Hazard Analysis and Critical Control Point System (PR/HACCP) was implemented to address with sanitation performance standards (Gamble, 2016). The *Salmonella* Verification program provided a solid foundation for selecting Salmonella as the test pathogen in this thesis.

1.2.3.1 *Salmonella* Food Borne Pathogen

Salmonella is the genus of several enteric, gram-negative, motile, facultative anaerobic species of bacteria (Taskila et al., 2012). To decrease *Salmonella* presence, bacterial cell membranes must be injured. Ways to injure the cells are: thermally treating (extreme cold or heat), drying, exposure to high acidity, and starving of nutrients.

When testing for *Salmonella* prevalence in meat product, the bacterial lag-time and injured cells must be taken into consideration to prevent lower/false negative results (Taskila et al., 2012). *Salmonella* injured cells take longer to recover and show colony presence of plates for verification detection. Buffered peptone water (BPW) is a nonselective enrichment media that allows sub-lethally injured cells to recover by providing the cells ample nutrients in an optimum environment.

This study utilized a strain *Salmonella* Typhimurium (STR) resistant to the antibiotic nalidixic acid, provided by the USDA Agricultural Research Service (ARS).

This strain of *Salmonella* is trackable due to its antibiotic resistance to nalidixic acid. By adding 100 ppm of nalidixic acid into all growth media, enrichment media, and dilution media, other bacteria present are eliminated while the highlighted strain is still present and viable (Gamble, 2016). Bacterial inoculation and sampling methods used in these studies utilized protocols from previously published literature (R. Buhr et al., 2003; R. Buhr et al., 2005).

1.2.4 Antimicrobial Intervention

To address pathogens during processing, antimicrobial chemical interventions are added to chillers to kill pathogens. Antimicrobial chemical interventions act as disinfectants. The combination of low temperatures and antimicrobials that injure or kill pathogens increases food safety. An anti-microbial either kills microorganisms or prevents microorganism growth (Bauermeister et al., 2008). The industry has developed a multi-hurdle approach in reducing pathogens to ensure meat quality. Chlorine was the staple antimicrobial for many years, but due to its fast half-life and skin bleaching effects it is not persistently used as the main antimicrobial (Wideman et al., 2016). There are numerous ones now in circulation in processing plants (Gamble, 2016). Bromine and Chlorine acids, acidified Sodium Chlorite, cetylpyridinium Chloride, and peracetic acid (PAA) are the most commonly applied antimicrobials (Gamble, 2016).

High organic presence can decrease the effectiveness of antimicrobials (Howarth, 2005). To ensure antimicrobial efficacy, all antimicrobials have a common step where they are applied. Antimicrobials have a limit for applied concentrations, determined by the USDA, to protect consumers from residual chemicals and ensure worker safety (Mion

et al., 2016). Though maximum concentrations are regulated, the type and applied concentrations of antimicrobials differ great among processing plants (Wideman et al., 2016).

1.2.4.1 Peracetic acid

This thesis investigation utilized peracetic acid (PAA) as the antimicrobial chemical intervention. PAA is a strong oxidizer that targets yeast and bacteria in the dairy product and meat processing field. There are many publications regarding PAA in poultry processing and its capabilities regarding *Salmonella* reductions (Bauermeister et al., 2008). PAA is a blend of acetic acid and hydrogen peroxide.

One reason PAA is commonly used in meat processing is because as an organic antimicrobial additive, it allows organic products to keep its product labeling after processing. PAA is highly water soluble and has an allowable concentration of 2,000 parts-per-million (ppm) during processing. This study utilized a 22% acetic acid and 4.8% hydrogen peroxide solution called MP-2C from Envirotech.

1.2.5 Ice Slurry

Water and energy consumption intensities cause the poultry industry problems due to increasing water and wastewater costs (Saravia et al., 2005). Regulations require meat products to be have under a certain pathogen concentration, to comply with pathogen standards processors use extensive water to these requirements.

Ice slurry can decrease water use with its faster carcass cooling rates and slower media heating rates (Piñeiro et al., 2004). Ice slurry is a heat transfer liquid known for its

rapid cooling capacity (Melinder, 2015). Ice slurry is a mixture of small ice particles (typically 1 mm or less in effective diameter) in a binary solution. The binary solution is made of water and a freezing point depressant (e.g., sodium chloride salt). The freezing point depressant not only lowers the freezing temperature of the ice slurry, but it also aids retained dispersion of smaller ice particles by demoting agglomeration (Melinder, 2015).

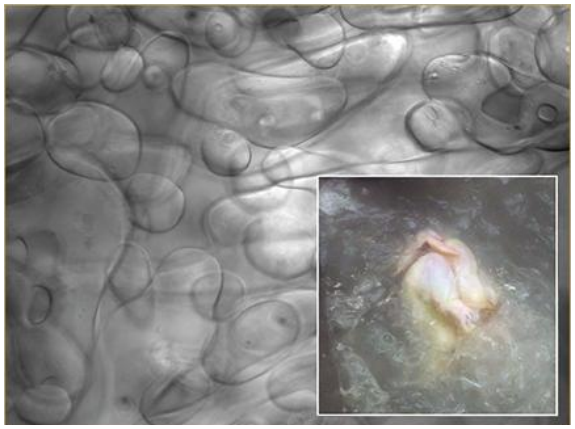

Figure 1.2.3 Ice slurry image captured under Nikon light microscope. Inset image is view of carcass immersed in ice slurry.

Ice slurry has various thermal-fluidic benefits. It has a large thermal capacity and low operating temperatures, providing better heat-sink qualities to that of traditional chilled water (Egolf, 2005). Additionally, the larger surface area per unit mass of the smaller characteristic length ice particles aids heat transfer and solid-to-solid contact between solid ice and heat source (e.g., poultry product). Ice slurry can be generated during off-peak electricity hours and stored overnight. When the chiller is ready for fill, the ice slurry (also termed "liquid ice") may be pumped for application. Ice slurry is also often used as a cooling agent for many applications. Fields that utilize ice slurry include building air conditioning, medical surgery, fire-fighting, food transport, vegetable

preservation, and the fishing sector (Kauffeld et al., 2010; Piñeiro et al., 2004). The presently reported application of ice slurry in the poultry industry was an application extension.

1.2.6 Ice Slurry Mechanical Abrasive Quality

Ice slurry has multiple benefits over chilled water with regard to thermal-fluidic features. The "liquid ice" also allows increased ice particle interactions with the carcass surface (Piñeiro et al., 2004). The ice slurry particles are thus hypothesized to distinctively aid in food safety given the antimicrobial benefits afforded by the added abrasion. The present thesis investigates the mechanical abrasion provided by ice slurry. We hypothesized that the abrasive quality of the ice particles disrupts or dislodge skin attached pathogens and allow antimicrobial penetration not seen with water chilling. An example is a two-sided sponge, the soft side does not scratch or harm the surface of dishes and glassware, the rough-scouring side and remove burnt and dried sauces. Ice slurry is like the scouring sponge side-, we hypothesize that ice slurry will enhance pathogen reduction during immersion chilling by mechanical-abrasive quality.

The freezing point depressant, also called an anti-agglomerate, keeps the slurry in a homogenous state and reduces ice agglomeration and media separation (Melinder, 2015). Salt brine solutions retain osmotic balance for products in a saline chilling medium. The salt used to generate the brine was an agricultural feed sodium chloride salt from Champion's Choice. Salt was added to room temperature water in a super saturated manner, the prepared brine averaged 26% salinity. When selecting the antimicrobial for immersion chilling experiments, a slower decay rate was a needed quality (Howarth,

2005). Brine solutions decrease chemical half-life. Peracetic acid was the best chemical intervention fitting these qualifications for ice slurry testing.

1.2.7 Ice Slurry Energy and Water Efficiency and Thermal Studies

The present thesis builds upon reported information from Ebony Rowe, a Master's graduate in Mechanical Engineering. Under the guidance of Dr. Comas Haynes, of the Georgia Tech Research Institute (GTRI) with joint appointment in Georgia Tech's Mechanical Engineering Department, Rowe and present author Richter conducted experiments testing for enhanced thermal cooling of poultry carcasses and STR pathogen reduction at seven treatment combinations. Thermal study results showed that in 20-minute immersion times, ice slurry provided an added temperature reduction average comparison to chilled water (Rowe, 2015).

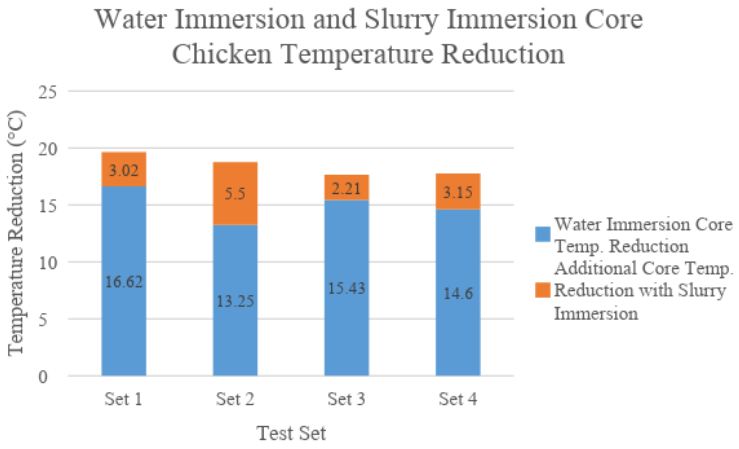

Figure 1.2.4 Ebony Rowe's Thermal Results. Orange shows the added thermal reductions seen with ice slurry over chilled water (blue).

CHAPTER 2. WHOLE CARCASS *SALMONELLA* REDUCTION AFTER 20-MINUTE IMMERSION CHILLING

This chapter was part of a multidisciplinary collaborative project. The three objectives were to determine the carcass thermal cooling, energy efficiency, and microbiological benefits of ice slurry for immersion chilling as opposed to chilled water. These three types of results are found in Ebony Rowe's thesis (Rowe, 2016). The biological data from Rowe's thesis and the present differ in reported concentration numbers and test analysis. This is due to the newly applied reporting of *Salmonella* Typhimurium concentration to allow for pathogen reduction comparison in Chapter 2, 3, and 4.

2.1 Introduction

The poultry industry consumes intense amounts of energy and water resources during processing and especially during the chilling phase. Traditional poultry chilling methods in the United States utilize cold water immersion chilling. Carcasses are immersed into chilled water with an antimicrobial intervention to achieve a temperature of ≤ 4 ◦C in the deep muscle region which may be the slowest cooling portion of the carcass.

The need for more sustainable resource is a common topic for the poultry industry. The Georgia Tech Research Institute (GTRI) has been validating ice slurry as a more financially feasible, faster carcass cooling, higher pathogen reducing, yet viable chilling

medium. These experiments involved thermal studies wherein the results are found in Rowe's thesis. This chapter presents comparisons and contrasts between chilled water and ice slurry media based upon reductions in pathogen concentration (cfu/mL) after immersion chilling.

Test subjects were ready-to-cook whole carcasses without the giblets (WOGs), used for rotisserie chickens. Each WOG was inoculated with 1 mL 10^6 cfu/mL of nalidixic acid resistant *Salmonella* Typhimurium (STR) and heated for two hours to mimic the temperatures of WOGs typically going into commercial chillers (~ 37 ◦C) and such heating additionally allow for bacteria time to acclimate to a new environment.

To simulate industry processing, a scaled-down auger chiller, on loan from the USDA-ARS (Athens, GA), was used in this study. In 20-minute immersion chilling trials, seven treatment combinations were investigated to examine trends of STR concentration reduction when compared pre- and post-chill. A treatment combination was defined by factors of: peracetic acid (PAA) ppm, Dosatron ratio (controls the salinity of the chilling media), and agitation level. The bacterial recovery of STR from the breast skin used a method from a previously published article (R. Buhr et al., 2003).

We hypothesize that ice slurry provides an abrasive/scrubbing quality that results in better pathogen reduction than the chilled liquid-water. The abrasive, scrubbing, quality can better dislodge bacteria or allow antimicrobial contact with pathogens.

2.2 Methods and Materials

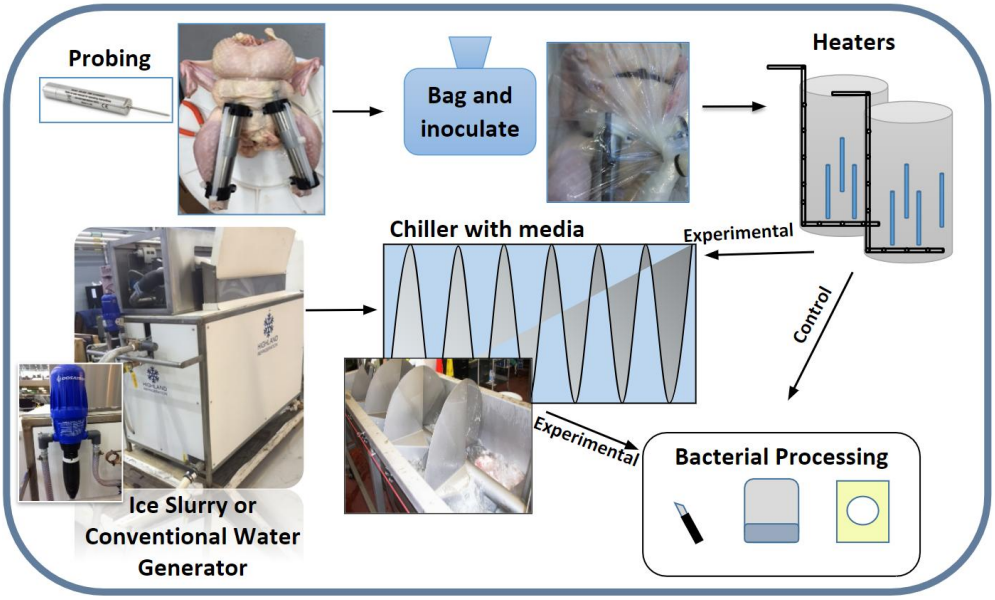

Figure 2.2.1 Experimental Process Flow, starting with inoculation of WOGs to bacterial recovery from breast skin rinse.

2.2.1 Preparations

For all experiments, media were prepared the Monday of the test week. This included: 3 1L 1% bottles of buffered peptone water (BPW, BD, Franklin Lakes, NJ), 2 1 L bottles of phosphate buffered saline (PBS, Fisher Scientific, Hampton, NH), and 1 0.5 L tryptic soy broth (TSB, BD, Franklin Lakes, NJ). Media were prepared according to company instructions and autoclaved. All scalpel handles, scalpel blades, tweezers, and glassware were sterilized via autoclave.

For fresh bacterial inoculation stock preparation 100 µL STR was aliquoted into 9.9 mL fresh TSB the day before all experiments and incubated at 37°C in an aerobic setting between 18 and 24 hours.

WOGs were collected the day of processing from a local processing plant, packed on ice in boxes of 12 and transported to the research lab. In the lab, all WOGs were

drained of liquids and individually bagged in Fisher brand 20x15 Sterile Stomacher bags and stored in the Ice Slurry Project refrigerator at 4°C until experimentation.

2.2.2 WOG Inoculation

Each test contained 24 individually bagged WOGs. Prior to STR inoculation, three WOGs or four WOGs were thermally probed to track heating and cooling temperatures of both the left and right breast meat (Figure 2.2.2). Probes tracked temperatures on a minute-by-minute basis for the duration of experiments. Probes were held in place using zip-ties (McMaster) and GTRI designed 3-D printed endcaps.

Figure 2.2.2 Thermally probed WOG using Thermoworks Thermadata Stainless Steel Temperature Logger with 2-inch probe inserted into the breast meat. Thermal probes were held in place using GTRI 3D printed endcaps and secured using 9-inch zip-ties.

The fresh overnight STR stock was assumed to be at a growth concentration of 10^9 cfu/mL. The STR stock concentration was confirmed using a spectrophotometer. A UV-Vis optical density of 600 was applied where a reading above 1.0 was considered 10^9

cfu/mL. The UV-Vis confirmation of 10^9 cfu/mL STR allowed proper dilution factors. The STR stock was serially diluted in PBS containing 100 ppm nalidixic acid to make a inoculation volume of 30 mL of 10^6 cfu/mL.

In a BSL-2 hood, each WOG was spiked with 1 mL 10^6 cfu/mL STR directly onto the center of the breast skin (Figure 2.2.3). The STR suspension was massaged into the breast skin for 15 seconds from outside the bag, and the bag was closed using a 4-inch zip-tie. The outside of all bags were wiped down with 70% isopropanol before being transported to the heating apparatus.

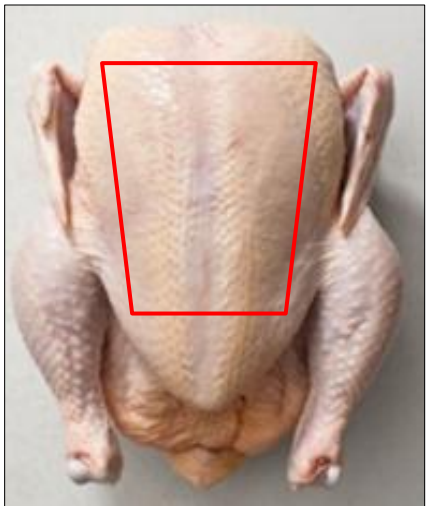

Figure 2.2.3 WOG inoculation and massage area, same area is excised via scalpel blade for bacterial recovery procedure.

2.2.3 WOG Heating

Each heating apparatus contained five 400W THEO heaters. Heaters were held in place with clips secured along the inside of a 44-gallon container. A 3/8 in. x 10 ft. perforated clear PVC tubing was connected to the blower on a shop-vacuum. The tubing was anchored via a metal weight that sat on the bottom of the containers (

Figure 2.2.4) to allow agitation/disruption of the WOGs during heating for forced convection. Heaters were filled with hot water to the internal brim. All THEO heaters were set to 35.5°C and turned on by being plugged into a surge protector. The heater apparatus ran for a minimum 30 minutes before WOGs were added. All 24 individually bagged inoculated WOGs were added to the heating apparatus at ~35.5 ◦C and heated with agitation for 2 hours. Heating aimed to warm the carcasses to mimic temperatures seen in industry post-evisceration/pre-chilling temperatures (37-38°C). Heating also allowed bacteria to acclimate to its new skin environment. The heaters did not exceed 12 WOG capacity for any of the tests.

Figure 2.2.4. Heating apparatus layout with THEO heaters, agitated with perforated airlines

2.2.4 Treatment Assignment

Post-heating, all WOGs were removed from their bags and drained for 3-4 minutes over wire racks in closed plastic bins. WOGs were then randomly assigned into

two groups: baseline (no chilling) and chiller intervention (ice slurry or chilled water). Of the 24 WOGs, 10 were assigned to the baseline group, while 14 were assigned to the chiller intervention group. The chiller intervention group received the thermally probed WOGs. The ten baseline WOGs were individually placed into a new stomaching bag and transported to the BSL-2 hood for bacterial recovery. The chiller intervention group was added to the chiller.

2.2.5 Chiller Intervention

Highland Refrigeration (Seattle, WA) provided the ice slurry generator machine used to fill the chiller with both media types (slurry and water). This machine first generated flake ice from a harvester, before cutting the thin ice sheets into fine ice particles. The ice particles were then combined with salty water to make the ice slurry media.

The level of sodium chloride in the salty water supply to the ice slurry generator was determined by the Dosatron unit. The Dosatron is a mixing unit = that combined brine and fresh water at set volumetric ratios (Figure 2.2.5). The Dosatron ratio is presented, in this thesis, as brine volume: fresh water volume or 1: XX.

Figure 2.2.5 Dosatron explanation of mixing ratios of brine: fresh water.

All experiments ran for twenty minutes chiller immersion in a 250-gallon screw-auger chiller on loan from USDA-ARS. The auger chiller was filled with either 125 gallons of chilled water or ice slurry (depending on the experimental intervention focus). All chilling media used to fill the chiller passed through the ice slurry machine, even when chilled water was the focus, to ensure the same gross salinity in the case of either medium. Tap water feeding into the slurry machine was from City of Atlanta.

2.2.5.1 Chilled Water Days

Once the screw auger chiller had the set amount of medium supplied, air agitation was connected and turned-on before Perasan MP-2C blend peracetic acid was added to the desired concentration (ppm). All 14 WOGs were added to the chiller and the 20-minute immersion chilling began. At the end of the 20 minutes, each WOG was removed using a large fishing net and allowed to drain/drip before being individually placed into new stomaching bags and transported for bacterial recovery. Probes were removed from WOGs before bacterial processing.

2.2.5.2 Ice Slurry Days

All subsequent procedures were the same as described above for water test days with the addition of measuring the ice slurry's ice fraction and salinity. The ice fraction was measured using a sample and French press, while a buoyancy probed was used to measure the salinity.

2.2.6 Bacterial Recovery

All bacterial recovery was performed in the Environmental lab's BSL-2 hood using approaches described in (R. Buhr et al., 2005). From each WOG, a breast skin sample was excised using sterile tweezers and scalpel. The excised skin sample, roughly 15 g, was placed into a smaller stomacher bag containing 50 mL buffered peptone water (BPW) containing 100 ppm nalidixic acid (NA). Skin samples were "stomached" for 60 seconds in a Masticator Lab-Blender (on loan for USDA-ARS). Each carcass skin sample's BPW solution was serially diluted to -10x, -100x, and -1000x in PBS+NA. All dilution factors were plated with two replicates, for a total of 6 plates per carcass. Plating utilized 3M Petrifilm aerobic plates where 1 mL was aliquoted, spread, and incubated at 37 ◦C for 22-24 hours.

2.2.7 Bacterial Analysis

A Doc-It colony counter and associated software, with a project specific devised colony identification template was used universally to count all STR Petrifilm plates. The dilution giving the best-fit counts were chosen, giving each WOG two plates to count.

The dilution factor displaying discernable counts between 3-300 CFU/mL was chosen. All sample plate count CFU/mLs were multiplied by its corresponding dilution factor. Each sample's breast skin recovered STR CFU/mL values were transformed to $\log_{10}$ cfu/mL before statistical analysis.

Data analysis is first presented by treatment combination replicates of the four groups and later combined replicates into one sample per treatment combination group. Data was analyzed using mean ± SD and Analysis of Variance (ANOVA) followed by Tukey HSD test a statistical significance determined at $P \leq 0.05$ by the JMP statistical software package.

2.2.8 Disposal/ Sterilization

All biological wastes and disposable lab consumables were placed into biohazard bags until pick-up from Georgia Tech Environmental Health and Safety (EHS). All other lab equipment were wiped down using a 10% Clorox bleach solution, and exposed to a bleach bucket for 30 minutes before being washed and dried. The lab floors were then mopped with the bleach solution.

After the experimental completion, 10% bleach was added to the chillers such that the concentration reached 10% bleach for at least 30 minutes. The chiller was then control drained. A water rinse was performed and followed by a spray degreaser foam (ZEP, Atlanta, GA). Before rinsing the foam, the chiller was scrubbed to disrupt any bacterial attachments to the sidewalls and then again rinsed with water.

2.3 Results and Discussion

This chapter was designed to discover ice slurry's pathogen reduction character when compared to chilled water. Each treatment combination was defined by three set factors: PAA ppm, Dosatron ratio, and air agitation level. The objective was to initially resolve trends regarding the optimum STR-reduction relationship between these factors. The best combination was defined as providing the largest added STR reduction of slurry when compared to water. Table **2.3.1** details the six treatment combinations tested.

To ensure STR reductions were due to immersion chilling and not outside factors, treatment combinations were completed in multiple replicates. For each treatment combination, the aim was to have three trials. A trial was defined by two immersion chilling experiments with one chilled water replicate and one ice slurry replicate. Each trial contains results of four groups.

1. Ice slurry
2. Ice slurry baseline
3. Chilled water
4. Chilled water baseline

Each trial had one chilled water replicate and one ice slurry replicate. A trial reports each immersion chilling experiment's reduction between a baseline group (pre-chill STR concentrations) and a chilled medium group (slurry or water); and each trial was completed in two days of the same week. Slurry and water tests were completed in a randomized order.

The agitation methods, 1x and 2x, are defined by the number of shop-vacuums pushing air through the auger chiller lines. Treatment combination 1 (TC1) was

performed during protocol refinement and did not provide any countable results. The STR reduction due to water immersion is presented by Δw, and reductions due to slurry are represented by Δs. The differences between slurry and water are represented by $\Delta s - \Delta w$, where positive values indicate higher slurry reductions and negatives indicate higher water reductions.

Table 2.3.1 Treatment combinations explored as defined by set Dosatron ratio, PAA concentration (ppm), and agitation levels in 20-minute immersion chilling times

Treatment Combinations Explored in WOG Experimentation				
Treatment Number	Treatment Combination	Dosatron Setting	PAA ppm	Agitation
TC2	1:14, 50 ppm, 1x	1:14	50	1x
TC3	1:18, 50 ppm, 1x	1:18	50	1x
TC4	1:14, 80 ppm, 1x	1:14	80	1x
TC5	1:14, 20 ppm, 1x	1:14	20	1x
TC6	1:18, 50 ppm, 2x	1:18	50	2x
TC7	1:14, 50 ppm, 2x	1:14	50	2x

Treatment combination 2 (TC2) included three trials/replicates. Each trial's average stomached breast skin recovered STR cfu/mL is reported in Table 2.3.2. In all three trials, slurry provided higher pathogen reduction, averaging 0.444 $\log_{10}$ more than chilled water. In the best trial, T2, slurry had 0.690 $\log_{10}$ increased pathogen reduction over water.

Treatment combination 3 (TC3) contained two completed trials. Each trial's average stomached breast skin recovered STR cfu/mL is reported in Table 2.3.2. In both trials, water provided higher reductions, average 0.239 $\log_{10}$ more than slurry. The slurry in this treatment was very thick, this was thought to be due to colder water temperatures feeding into the slurry machine from seasonal influences and lower salinity (due to high ice fraction and great Dosatron ratio). The slurry very quickly stratified into a distinct ice

layer above the liquid portion. The ice slurry may have lost the hypothesized abrasive quality due to the loss of a homogenous nature.

Treatment combination 4 (TC4) contained three trials. Each trial's average stomached breast skin recovered STR cfu/mL is reported in Table 2.3.2. In two trials, slurry provided higher reductions than water, averaging 0.305 $\log_{10}$ more from all three trials. In the best trial, T3, slurry provided 0.601 $\log_{10}$ more pathogen reduction than chilled water.

Treatment combination 5 (TC5) contained two trials. Each trial's average stomached breast skin recovered STR cfu/mL is reported in Table 2.3.2. In both replicates ice slurry provided higher reduction than water, averaging 0.276 $\log_{10}$ higher. In the highlight trial, T1, slurry provided 0.533 $\log_{10}$ better STR reduction.

Treatment combination 6 (TC6) contained three trials. Each trial's average stomached breast skin recovered STR cfu/mL is reported in Table 2.3.2. the two corresponding replicates, ice slurry averaged a 0.130 log better reduction compared to water. Results show slightly differences in STR reductions between the chilling mediums. To keep the ice slurry more uniform with higher ice fractions (addressing issues from TC3), this treatment combination used a double (2x) agitation application where two attached shop vacuums push air through the chiller lines. The ice slurry stayed more uniform than seen in TC3. The doubled compressed air feeding into the chiller heated the media faster so that the ice portion melted faster than previous treatments. The melting/loss of ice particles eliminates any of the hypothesized abrasive pathogen disruption qualities. Faster melt rates and slight particles agglomeration could impact the pathogen reduction capabilities.

Treatment combination 7 (TC7) contained four trials. The average stomached breast skin recovered STR cfu/mL is reported in Table 2.3.2. Water reductions were between 0.605 – 0.893 $\log_{10}$; slurry reductions were between 0.696 – 1.436 $\log_{10}$. In the three corresponding replicates, slurry provided higher STR reductions by an average 0.178 $\log_{10}$ more than water. In TC6 and TC7, more slurry immersion replicates were performed than water experiments. This was to build a larger sampling size to support the hypothesized scrubbing effect.

Table 2.3.2 Treatment combinations presented by trials with average mean STR reductions from baseline groups with associated P-values.

Treatment Combination 2				
	Δ_W	$P =$	Δ_S	$P =$
T1	0.826	<.0001	0.770	<.0001
T2	0.425	0.230	1.115	<.0001
T3	0.404	0.240	0.705	0.001
T4	0.834	<.0001	1.176	<.0001
Treatment Combination 3				
	Δ_W	$P =$	Δ_S	$P =$
T1	0.823	<.0001	0.684	<.0001
T2	0.978	<.0001	0.639	0.001
Treatment Combination 4				
	Δ_W	$P =$	Δ_S	$P =$
T1	0.752	<.0001	1.127	<.0001
T2	0.812	<.0001	0.753	<.0001
T3	0.367	0.180	0.968	<.0001
Treatment Combination 5				
	Δ_W	$P =$	Δ_S	$P =$
T1	0.870	<.0001	0.890	<.0001
T2	0.352	0.004	0.885	<.0001
Treatment Combination 6				
	Δ_W	$P =$	Δ_S	$P =$
T1	0.709	<.0001	0.990	<.0001
T2	0.968	<.0001	0.947	<.0001
T3			0.961	<.0001
Treatment Combination 7				
	Δ_W	$P =$	Δ_S	$P =$
T1	0.625	0.001	0.696	0.000
T2	0.605	0.001	0.922	<.0001
T3	0.893	<.0001	1.038	<.0001
T4			1.437	<.0001

Comparisons between all treatment combinations from combined trials for all four groups are presented in Table 2.3.3 where mean and standard deviation cfu/mL are given. In all treatment replicates there has a high degree of variability in the cfu/mL of recovered STR. SD variability was highest when replicates were combined as a single sample set. All baseline group concentrations varied between each trial and impacted SD and p-value of combined trials.

Table 2.3.3 Stomached breast skin rinse recovered STR cfu/mL by treatment combination. All trials/replicates were combined into one representative sample set per group.

Treatment combination and groups		STR $\log_{10}$ cfu/mL	
TC2	1:14, 50 ppm, 20 min, 1x		
	Ice slurry baseline	5.449	± 0.328
	Ice slurry	4.273	± 0.413
	Chilled water baseline	5.549	± 0.302
	Chilled water	4.988	± 0.421
TC3	1:18, 50 ppm, 20 min, 1x		
	Ice slurry baseline	5.424	± 0.189
	Ice slurry	4.761	± 0.440
	Chilled water baseline	5.660	± 0.272
	Chilled water	4.748	± 0.316
TC4	1:14, 80 ppm, 20 min, 1x		
	Ice slurry baseline	5.492	± 0.358
	Ice slurry	4.521	± 0.370
	Chilled water baseline	5.367	± 0.372
	Chilled water	4.724	± 0.362
TC5	1:14, 20 ppm, 20 min, 1x		
	Ice slurry baseline	5.813	± 0.330
	Ice slurry	4.926	± 0.333
	Chilled water baseline	5.239	± 0.309
	Chilled water	4.639	± 0.598
TC6	1:18, 50 ppm, 20 min, 2x		
	Ice slurry baseline	5.414	± 0.257
	Ice slurry	4.439	± 0.338
	Chilled water baseline	5.25	± 0.247
	Chilled water	4.412	± 0.252
TC7	1:14, 50 ppm, 20 min, 2x		
	Ice slurry baseline	5.246	± 0.339
	Ice slurry	4.222	± 0.549
	Chilled water baseline	5.303	± 0.323
	Chilled water	4.595	± 0.470

Table 2.3.4 ANOVA analysis of stomached breast skin recovered STR log10 cfu/mL by treatment combination

ANOVA Tukey HSD			
Treatment	Δ	$\log_{10}$ cfu/mL	P-value
TC2	Δ_S	0.941	<.0001*
	Δ_W	0.622	<.0001*
	$\Delta_S\Delta_W$	0.319	0.040*
TC3	Δ_S	0.661	<.0001*
	Δ_W	0.900	<.0001*
	$\Delta_S\Delta_W$	-0.239	0.999
TC4	Δ_S	0.949	<.0001*
	Δ_W	0.644	<.0001*
	$\Delta_S\Delta_W$	0.305	0.909
TC5	Δ_S	0.888	<.0001*
	Δ_W	0.611	0.0001*
	$\Delta_S\Delta_W$	0.276	0.752
TC6	Δ_S	0.966	<.0001*
	Δ_W	0.838	<.0001*
	$\Delta_S\Delta_W$	0.127	1.000
TC7	Δ_S	1.023	<.0001*
	Δ_W	0.708	<.0001*
	$\Delta_S\Delta_W$	0.316	0.015*

The overall pathogen difference of slurry reduction and water reduction are presented in Table 2.3.3. TC2 and TC7 had statistically significant differences in $\Delta_S\Delta_W$ ($P \leq 0.05$), with average 0.319 and 0.316 $\log_{10}$ added slurry reduction. The optimum treatment factors appear to be PAA 50 ppm with Dosatron ratio 1:14. The air agitation levels had to be adjusted to keep a uniform ice slurry or less to minimal added pathogen reduction was found.

Examples of non-ideal slurry phenomena occurred with TC3 and TC6. Dosatron ratio of 1:18 may promote faster agglomeration of the ice particles. The slurry in TC3 quickly separated so that 1x agitation level could not penetrate through the top ice layer, WOGs were denied slurry interactions as they were lodged under the ice layer. TC6

displayed a more uniform slurry mix with the application of the 2x agitation method but melted the ice particles so that none were present at the end of the 20-minutes. Issues with ice slurry consistency yielded similar reductions between slurry and water (P-value > 0.90) in TC3 and TC6. TC4 and TC5 may not have been statistically significant but presented a trend for slurry's potential to provide enhanced pathogen reduction.

Sources of experimental result variation could be due to seasonal city water temperatures feeding into the ice slurry generator, processing plant added chemical concentrations and chilling lengths during carcasses processing, WOG size differences and total fat composition, and nature of bacterial presence on skin after heating.

For more standardized results, all skin weights need to be weighed and trimmed to a defined range. In these treatment combinations, each WOG averaged 12.5 gallons of chilling medium. This amount is more than is used in industry chilling, average 5 L/bird, and could allow for higher pathogen reductions with "cleaner" water. Pre- and post-chill WOG weights were initially recorded as part of these test to take water weight gain amounts. Already processed meat product was thought to not reflect water uptake properties seen in industry as it had already undergone processing.

CHAPTER 3.

INITIAL INVESTIGATION OF ICE SLURRY'S ABILITY TO AID IN *SALMONELLA* TYPHIMURIUM REDUCTIONS DURING POULTRY WING-PARTS IMMERSION CHILLING

3.1 Introduction

The Center for Disease Control reported that between 2012 and 2013, 634 people were reportedly infected with a strain of *Salmonella* Heidelberg. The source of infection was due to mishandling of California's Foster Farms brand contaminated packaged chicken (Prevention, 2014). Prior to this outbreak, the USDA pathogen verification system focused on inspecting whole carcass meat product. Post-outbreak, the USDA-FSIS released a press release, in January 2015, regulating pathogen presence in poultry parts and ground meat product (FSIS, 2016). The USDA proposed new standards to reduce *Salmonella* and *Campylobacter* in chicken parts, ground chicken, and ground turkey. Many plants now apply a post-dip step to deal with parts contamination. The post-dip method adds meat products into chilled water holding high antimicrobial concentrations (up to 2,000 ppm for peracetic acid) that cover the products for 2-3 minutes (Gamble, 2016).

This chapter addresses poultry parts contamination with specific focus upon whole wing parts. Ready-to-cook, processed wings "spiked" with a Nalidixic acid (NA) antibiotic resistant strain of *Salmonella* Typhimurium (STR) were studied to see the

impact of the chiller medium pathogen reduction capabilities. Four treatment combinations were tested, wherein a treatment combination was defined by factors of immersion length (10 or 20 minutes), Dosatron setting (ratio of brine: "fresh" water for the ice slurry generator), and peracetic acid (PAA) ppm. Each treatment compared chilled water and ice slurry chilling media impact upon STR reductions.

All wings were dipped into a 10^6 STR cfu/mL solution, heated for 60 minutes at 35°C (to allow for bacterial attachment), and immersion chilled within internally fabricated microchillers. Two microchillers were constructed so that ice slurry and chilled water immersion experiments could occur concurrently. The microchillers each contained 60 L of chilling medium, with air agitation lines feeding from the bottom of the microchillers. Experiments occurred from August 2016 to December 2016 at the GTRI Food Processing Technology Building.

It was again hypothesized that ice slurry provides an abrasive sheer quality that increases pathogen reductions compared to traditional chilled water.

3.2 Methods and Materials

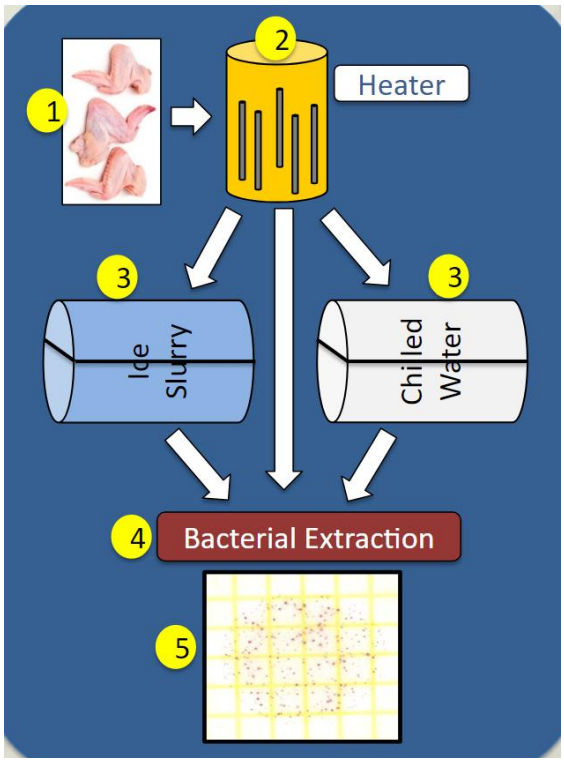

Figure 3.2.1. Experimental process flow for wings study. Experiments start with wing dip in STR and end with bacterial recovery.

3.2.1 Preparations

For all experiments, all media were prepared the Monday of the test week. This included: five 1L bottles of BPW, seven 1 L bottles of PBS, and one 0.5 L TSB.

For fresh bacterial inoculation STR stock, 100 µL *Salmonella* Typhimurium strain (resistant to nalidixic acid) was aliquoted into 9.9 mL fresh tryptic soy broth the day before all experiments. The samples incubated at 37˚C in an aerobic setting for 18 hours to 24 hours.

Processed wings were donated by a local processor the day of testing and packed on ice for transport to the experimental location. In the lab, wings were placed onto a drip

tray to air dry for 5 minutes and individually bagged and stored in the Ice Slurry Project refrigerator at 4°C until experimentation.

3.2.2 Wings Inoculation

Overnight STR growth of 10^9 cfu/mL was confirmed using UV-Vis OD600 with any reading above 1.0 considered 10^9 cfu/mL. STR was serially diluted in PBS containing 100 ppm nalidixic acid to reach a total volume of 6 L of 10^6 cfu/mL. Wings were air dried on a drip plate (VWR, Randor, PA) for 2 minutes before being added to a dip-bath for 5 minutes. The dip-bath was composed of 6 L of PBS and STR concentration of 10^6 CFU/mL. After the dip-bath, the wings were again air dried for two minutes and individually bagged for heating.

3.2.3 Wing Heating

Each heating apparatus contained five 400W THEO heaters slip-secured along the inside of a 44-gallon container filled with hot water. A propeller was inserted near the bottom of the container to agitate the water for uniform heating. As described previously, heaters were filled with hot water and turned on, and the heater apparatus sat for at least 30 minutes before any bagged wings were added. All individually bagged wings were added to heaters with water at ~35.5°C for 90 minutes.

3.2.4 Treatment assignment

Post-heating, all wings were removed from their bags and allowed to air dry for 2 minutes on drain racks. The 36 wings were then randomly assigned into three groups:

12/batch baseline (no chilling), 12/batch chilled water (control), or 12/batch ice slurry (alternative). Baseline wings were added to a new bag and transported to bacterial processing, while the chiller intervention wings were added to their assigned chiller at a predetermined treatment combination. A treatment combination was defined by factors in:

1. Dosatron ratio (brine: fresh water)
2. PAA ppm
3. Immersion chilling length (minutes)

3.2.5 Chiller Intervention Treatments - -Same PAA and Dosatron

All media used to fill the microchillers passed through the ice slurry machine. Water feeding into the slurry machine was from City of Atlanta. The microchillers were filled with 60 L of assigned medium, respectively.

Figure 3.2.2 GTRI Microchillers designed for wing-part chilling

3.2.5.1 Chilled Water Microchiller

The microchiller was filled with 60L of chilled water from the Highland Refrigeration machine, and the agitation method was started. Perasan MP-2C blend PAA was added to the desired concentration in ppm. The wings were added to the chillers and the immersion timer was started. Once immersion times were completed, the wings were removed by tongs, air dried on a drip plate for 2-minutes, and placed in a new sterile stomaching bag. Wing bags were then transported to the BSL2 hood for bacterial extraction.

3.2.5.2 Ice Slurry Microchiller

The microchiller was filled with 60L of ice slurry from the Highland Refrigeration machine and the agitation method was started. The ice fraction was measured a sample of slurry using a French press, while a buoyancy probed was used to measure the salinity. The Perasan MP-2C blend PAA was added to the desired concentration (ppm). The wings were added, and the immersion timer started.

Once the immersion time was completed, the wings were removed with tongs, air dried on a drip plate for 2-minutes, and placed in a new sterile stomaching bag. All bagged wings (3 * 12/batch) were transported to bacterial recovery in the BSL-2 hood.

3.2.6 Bacterial Recovery and Statistics

All bacterial recovery was performed in the Environmental Lab's BSL2 hood. Each wing, in its new stomacher bag, had 100 mL BPW added. Wings were "stomached"

for 60 seconds in a Masticator Lab-Blender. Post-stomaching, each wing's BPW STR rinsate was serially diluted to -10x, -100x, and -1000x in PBS+NA. All dilution factors were plated with two replicates, for a total of 6 plates per sample. Plating utilized 3M Petrifilm aerobic plates where 1 mL was aliquoted and spread, and the plates were incubated at 37°C for 22-24 hours.

After incubation, plates were counted using a Doc-It colony counter and associated software, a developed colony identification template was used comprehensively between all STR plate counts. The dilution giving the best-fit counts was chosen, giving each wing two plates. The dilution factor displaying discernable counts between 3-300 CFU/mL was chosen. Each sample's plate counts (cfu/mL) were multiplied by its corresponding dilution factor. Each sample was represented by two plates that were averaged together to represent that wing's STR concentration. All wing counts were cast in terms of $\log_{10}$ cfu/mL before statistical analysis.

Data analysis is first presented by treatment combination replicates of the four groups and later combined replicates into one sample per treatment combination group. Data was analyzed using mean ± SD and Analysis of Variance (ANOVA) followed by the Tukey HSD test at a statistical significance determined at $P \leq 0.05$. Analyses were done using the JMP statistical software package.

3.2.7 Disposal/ Sterilization

All biological waste and disposable lab consumables were placed in biohazard bags until pick-up from Georgia Tech EHS. All other lab equipment was sanitized as previously described in Chapter 2.

3.3 Results and Discussion

This protocol was designed as a scaled-down model of the WOGs protocol conveyed in Chapter 2. Scaling down allowed faster determination of the optimum treatment combination factors while conserving resources. Four treatment combinations were tested in this chapter (Table 3.3.1). Treatment combinations were defined by factors of: Dosatron ratio, PAA ppm, and immersion length (minutes).

Results are presented by trials/replicates from each of the four micro-chiller treatment combinations (MCTC#). For each trial, slurry and water immersion groups shared the same baseline group. A trial was completed in one day, where the slurry wing group and water wing group micro-chillers ran concurrently. The STR recovered from the "stomached" wing rinse due to immersion chilling falls under its corresponding media type: water or slurry. The STR reduction from baseline due to water immersion is presented by Δw, and reductions due to slurry are presented by Δs. The added STR differences in water and slurry are presented by $\Delta s - \Delta w$, where positive values indicate higher slurry reductions and negatives indicate higher water reductions.

Table 3.3.1 Treatment combinations explored in wing immersion studies. Treatment combination defined by Dosatron setting, PAA ppm, and immersion chilling length

Treatment Number	Treatment Combination	Dosatron Setting	PAA ppm	Length (Minutes)
MCTC1	1:32, 50 ppm, 20 min	1:32	50	20
MCTC2	1:32, 50 ppm, 10 min	1:32	50	10
MCTC3	1:14, 50 ppm, 10 min	1:14	50	10
MCTC4	1:14, 50 ppm, 20 min	1:14	50	20

Micro-chiller treatment combination 1 (MCTC1) contained three trials. The average $\log_{10}$ STR cfu/mL recovered from the wing sample is detailed in Table 3.3.2 by mean ± SD of each replicate group. In all replicates, slurry provided higher reductions than water, averaging 0.250 $\log_{10}$ greater reduction than chilled water. In the highlight trial, T2, slurry provided an additional 0.308 log reduction over chilled water.

Table 3.3.2 Micro-chiller treatment combination 1: Dosatron 1:32, 50 PAA ppm, for 20-minute immersion

Dosatron 1:32, 50 ppm PAA, for 20 minutes						
	Salmonella Typhimurium $\log_{10}$ cfu/mL					
MCTC1	Baseline	Water	Slurry	*Δw*	*Δs*	*Δs-Δw*
T1	7.291 ± 0.153	6.248 ± 0.174	5.992 ± 0.182	1.043	1.299	0.256
T2	7.330 ± 0.094	6.137 ± 0.121	5.829 ± 0.259	1.193	1.501	0.308
T3	7.226 ± 0.110	6.227 ± 0.128	6.040 ± 0.176	0.998	1.185	0.187
Avg.	7.282	6.204	5.954	1.078	1.329	0.250

Micro-chiller treatment combination 2 (MCTC2) contained three trials. The average $\log_{10}$ STR cfu/mL recovered from the wing sample is detailed in Table 3.3.3 by mean ± SD of each replicate group. In all replicates slurry provided higher reductions, averaging 0.151 $\log_{10}$ more reduction than water. In the highlight trial, T2, slurry yielded 0.232 $\log_{10}$ added reduction over chilled water.

Table 3.3.3 Micro-chiller treatment combination 2: Dosatron 1:32, 50 ppm PAA, for 10-minute immersion

Dosatron 1:32, 50 ppm PAA, for 10 minutes						
	Salmonella Typhimurium $\log_{10}$ cfu/mL					
MCTC2	Baseline	Water	Slurry	*Δw*	*Δs*	*Δs-Δw*
T1	7.126 ± 0.135	5.935 ± 0.252	5.894 ± 0.238	1.191	1.232	0.042
T2	6.993 ± 0.260	5.971 ± 0.066	5.739 ± 0.206	1.022	1.255	0.232
T3	7.053 ± 0.211	5.894 ± 0.221	5.713 ± 0.177	1.160	1.340	0.180
Avg.	7.060	5.953	5.816	1.124	1.276	0.151

Micro-chiller treatment combination 3 (MCTC3) contained four trials. The average $\log_{10}$ STR cfu/mL recovered from the wing sample is detailed in Table 3.3.4 by mean ± SD of each replicate group. In all four trials, slurry provided higher STR reduction than water, averaging 0.206 log more. In the highlight trial, T4, slurry provided an additional 0.319 log reduction over chilled water.

Table 3.3.4 Micro-chiller treatment combination 3: Dosatron 1:14, 50 ppm PAA, for 10-minute immersion

Dosatron 1:14, 50 ppm PAA, for 10 minutes						
	Salmonella Typhimurium $\log_{10}$ cfu/mL					
MCTC3	Baseline	Water	Slurry	*Δw*	*Δs*	*Δs-Δw*
T1	7.271 ± 0.186	5.820 ± 0.234	5.778 ± 0.243	1.452	1.493	0.042
T2	6.479 ± 0.185	5.714 ± 0.271	5.441 ± 0.256	0.765	1.039	0.273
T3	5.504 ± 0.202	4.521 ± 0.428	4.330 ± 0.334	0.983	1.175	0.191
T4	6.814 ± 0.141	5.737 ± 0.152	5.418 ± 0.152	1.078	1.397	0.319
Avg.	6.418	5.352	5.183	1.069	1.276	0.206

Micro-chiller treatment combination 4 (MCTC4) contained three trials. The average $\log_{10}$ STR cfu/mL recovered from the wing sample is detailed in Table 3.3.5 by mean ± SD of each replicate group. In all trials, slurry provided higher pathogen reduction, averaging 0.252 $\log_{10}$ more. In the highlight trial, T3, slurry provided an additional 0.354 log reduction over chilled water.

Table 3.3.5 Micro-chiller treatment combination: Dosatron 1:14, 50 ppm PAA, for 20-minute immersion

Dosatron 1:14, 50 ppm PAA, for 20 minutes						
	Salmonella Typhimurium $\log_{10}$ cfu/mL					
MC#4	Baseline	Water	Slurry	*Δw*	*Δs*	*Δs-Δw*
R1	6.479 ± 0.185	5.572 ± 0.263	5.305 ± 0.292	0.907	1.174	0.267
R2	5.504 ± 0.202	4.457 ± 0.370	4.320 ± 0.289	1.047	1.184	0.137
R3	6.814 ± 0.141	5.725 ± 0.196	5.372 ± 0.266	1.089	1.443	0.354
Avg.	5.992	5.015	4.813	1.015	1.267	0.252

For Table 3.3.6 the averaged $\log_{10}$ CFU/mL from all microchiller treatment combinations show the high degree of standard deviation in MCTC3 and MCTC4. Data analysis did not use a correctional test to fit the baseline concentrations. Future statistical analysis will be performed to better fit the data.

Table 3.3.6 Comparing treatment combinations from averaged replicate values

Treatment combination and groups		STR $\log_{10}$ cfu/mL	
1	1:32, 50 ppm, 20 min, 1x		
	Baseline	7.279	± 0.126
	Ice slurry	5.963	± 0.216
	Chilled water	6.204	± 0.146
2	1:32, 50 ppm, 10 min, 1x		
	Baseline	7.053	± 0.212
	Ice slurry	5.789	± 0.218
	Chilled water	5.927	± 0.204
3	1:14, 50 ppm, 10 min, 1x		
	Baseline	6.517	± 0.680
	Ice slurry	5.242	± 0.604
	Chilled water	5.438	± 0.614
4	1:14, 50 ppm, 20 min, 1x		
	Baseline	6.266	± 0.591
	Ice slurry	4.999	± 0.560
	Chilled water	5.251	± 0.638

Table 3.3.7 Reductions due to chilling media for all four treatment combinations

Treatment #	Δ	$\log_{10}$ cfu/mL	P-value
MCTC1	Δ_S	1.316	<.0001*
	Δ_W	1.078	<.0001*
	$\Delta_S\Delta_W$	0.250	0.789
MCTC2	Δ_S	1.263	<.0001*
	Δ_W	1.126	<.0001*
	$\Delta_S\Delta_W$	0.137	0.999
MCTC3	Δ_S	1.276	<.0001*
	Δ_W	1.079	<.0001*
	$\Delta_S\Delta_W$	0.197	0.838
MCTC4	Δ_S	1.267	<.0001*
	Δ_W	1.015	<.0001*
	$\Delta_S\Delta_W$	0.252	0.713

The STR reductions by media type are displayed in Table 3.3.7, and it shows that MCTC1 was the only statistically significant ($p < 0.0001$) treatment combination difference in Δ_S-Δ_W. Because of its statistical significance, MCTC1 was the most impacting treatment combination for this phase of experimentation, and this combination is defined by Dosatron 1:32, 50 PAA ppm, for 20-minute immersion chilling. With Dosatron 1:32, the ice slurry was very thick in quality. The thicker the slurry, there is more potential for pathogen scrubbing/abrasion. This is so long as neither stratification nor immobility become confounding phenomena. MC4 provided about the same Δ_S-Δ_W average STR reduction as MC1 (0.252 $\log_{10}$ vs. 0.250 $\log_{10}$), but it was not resolved to be statistically different. A cause for MC4 being deemed insignificant, is thought to result from significant variability effects.

For this chapter, wings averaged 6 L of chilling media per wing, which is much higher than seen in industry. Future tests would include larger numbers of wings into chilling medium groups to reducing the amount of media per wing.

Addition of the microchiller's airlines mimicked agitation methods utilized in Chapter 2. The 60 L of chilling medium heated much faster than the 125-gallon amount used in Chapter 2. The same shop vacuums from Chapter 2 were used as the agitation method in the microchillers. The shop vacuum feeding air into the agitation lines of the microchiller was too powerful. The increased media heating resulted in the ice slurry completely melting before the finish of the 20-minute immersion chill. When the ice slurry melts, we are losing the hypothesized abrasive benefits and pathogen reduction. Further protocol refinement is needed to resolve the melting issue.

The agitation methods originated with a propeller application but were abandoned, because the wings would suction to the back of the propeller and not dislodge until the propeller was turned-off. The propeller agitation yielded no differences in pre- and post-chill STR counts. The propeller issues led to the development of added airlines to the bottom of the microchillers (Figure 3.2.2).

Ice slurry chilling medium can provide higher pathogen reduction over traditional methods. These results continued to support the plausibility of conditions wherein abrasive/scrub benefits of using ice slurry manifest, yet there were confounding effects that are explained and addressed in the ensuing chapter.

CHAPTER 4. ICE SLURRY'S ABILIY TO AID IN *SALMONELLA* TYPHIMURIUM REDUCTION DURING POULTRY WING-PARTS IMMERSION CHILLING WHILE CONTROLLING FOR SALINITY AND PERACETIC ACID CONCENTRATIONS

4.1 Introduction

Ice slurry is composed of ice particles in a binary liquid composed of salt and water (Egolf, 2005). The Highland Refrigeration ice slurry machine determines solution salinity with the Dosatron component. The Dosatron sets the ratio of brine to fresh water that is combined for the appropriate salinity salt water to be the feedstock into the slurry generator. The brine is made by adding Champion's Choice Mix'n'Fine agricultural feed sodium chloride salt into "pure" water in a supersaturated manner (salt still remains after dissolving). Salt is a vital part of ice slurry as it aids in demoting agglomeration and keeping the small ice particles dispersed within the slurry.

The ice slurry's salt components are only significantly found in the liquid-water portion; i.e., minimal amounts are found within the sold ice particles. Similarly, once the antimicrobial peracetic acid (PAA) is added to the ice slurry, the PAA is only anticipated in significant quantities within the liquid-water portion. When the same "gross" PAA concentration is added to both ice slurry and chilled water (i.e., based upon the total volumes of the respective chiller media), the liquid portion of the slurry holds higher concentrations of PAA. The ice fraction acts as a volume displacement influence that

increases salinity and PAA liquid concentrations, which could result in better pathogen reduction.

To support our hypothesis of ice slurry providing a mechanical abrasive character, initial PAA concentration and salinity in the liquid portions of the chilling media had to be controlled for consistency. Accordingly, to negate confounding effects of different liquid-phase concentrations of salt and PAA in the *Salmonella* pathogen reduction study, "effective setting" calculations to regulate for initial concentrations were developed.

At the beginning of experiments, ice slurry and chilled water needed to mimic each other in PAA and salinity concentrations. First, a set PAA concentration was defined for chilled water 60 liter testing volume. From the chilled water's defined PAA concentration, the measured ice fraction of slurry resulted in a proportional reduction of the PAA volume added to slurry. Ice fraction allowed determination of liquid-water volume. Additionally, once the salinity of the slurry's liquid water phase was measured by a buoyancy probe, the Dosatron ratio was adjusted (based upon an equation) for the chilled water to generate the same salinity levels.

The associated "effective settings" experiments used prior methods developed for the microchiller with the addition of two adjustment calculations. Additionally, air agitation displacement plates (

Figure 4.2.1) were physically incorporated for better flow management. We continue to hypothesize that ice slurry provides an abrasive scrubbing quality that increases *Salmonella* reductions during immersion chilling compared to traditional chilled water applications. The presently reported methodology controlled for two confounding

variables that are known to affect pathogen reduction. Controlling for salinity and PAA initial concentrations within the liquid phase allowed more focused attention upon the ice slurry scrubbing effect regarding pathogen reductions.

4.2 Methods and Materials

"Methods and Materials" followed that of the previous chapter with the three additions:

1. Matching initial PAA concentrations (ppm) in slurry and water's liquid-water portion using the calculation below.

$$Slurry\ PAA\ reduce = (60L - (60L * \%\ ice\ fraction)) * water\ set\ ppm$$

2. Matching initial salinity in slurry and water's liquid-water portion using the Dosatron ratio adjustment calculation below.

$$Water\ dosatron = slurry\ dosatron * (1 - \%\ ice\ fraction)$$

3. Agitation displacement plates seen below in Figure 4.2.1

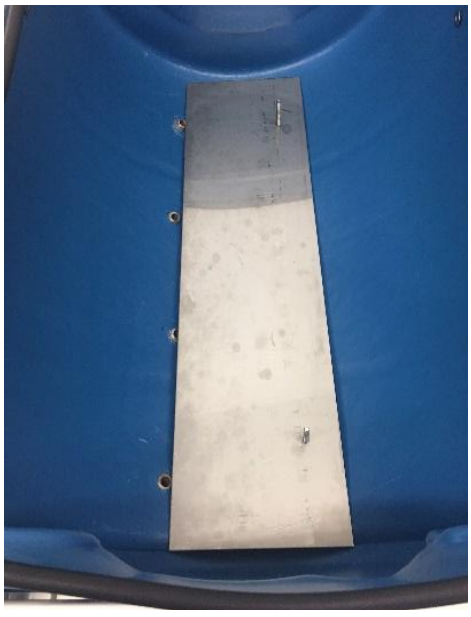

Figure 4.2.1 Air agitation displacement plate that reduced rate of media heating

For each trial the ice slurry microchiller was generated before the chilled water. The ice fraction and salinity of the ice slurry had to be measured to calculate the amount of PAA to add to the slurry, and it allowed for adjustment of the Dosatron setting for the chilled water microchiller tests.

4.3 Results and Discussion

Salt and PAA concentrations impact pathogen reductions. These results show a trend of ice slurry's greater capacity for *Salmonella* reduction after immersion chilling, even when controlling for initial salinity and PAA concentrations. Four treatment combinations were tested in this chapter (Table 4.3.1). Treatment combinations were defined by factors in Dosatron ratio, PAA ppm, and immersion length (minutes).

Results are presented by each effective setting treatment combination. The three groups of the treatment combinations are presented by trial mean ± SD. For each trial, slurry and water groups shared the same baseline group. The baseline group received no chilling and provided representative pre-chill STR concentrations. A trial was completed in one day, where the slurry group and water group microchillers ran concurrently. The STR reduction due to water immersion chilling (Δw) calculated by comparing baseline and chilling groups wing-rinse recovered STR concentrations. Similarly, reductions due to slurry immersion chilling (Δs) calculated by comparing baseline to chilling group wing-rinse STR concentrations. The STR reduction differences between water and slurry are presented by $\Delta s - \Delta w$, where positive values indicate higher reductions due to ice slurry and negative values indicate higher reductions with water.

Table 4.3.1 Treatment combinations tested in effective settings wing chilling experiments. The Dosatron ratio was adjusted for chilled water depending on slurry salinity. The amount of PAA added to slurry was reduced depending on the ice fraction.

Effective Settings Microchiller Wing Treatments				
ESTC#	Slurry Dosatron	PAA ppm in Liquid-Water at t=0	Chilling (min)	Media Volume
ESTC1	1:22	50	10	60 L
ESTC2	1:30	50	20	60 L
ESTC3	1:22	50	20	60 L
ESTC4	1:22	30	10	60 L

Effective setting 1 (ESTC1) included five trials. ESTC1 explored 20-minute immersion length, slurry Dosatron ratio of 1:22, and initial PAA concentration of 50 ppm. STR average (mean) $\log_{10}$ cfu/mL values and respective standard deviations recovered from the stomached wing rinse are reported in Table 4.3.2. In all five trials, slurry provided higher STR reduction over water, averaging 0.368 $\log_{10}$ higher from all five trials. In the most distinguishing trial, T2, slurry yielded 0.548 $\log_{10}$ added reduction over chilled water.

Table 4.3.2 Effective Setting 1: Slurry Dosatron 1:22, 50 ppm PAA water, for 20 minutes of immersion

	Salmonella Typhimurium $\log_{10}$ cfu/mL					
ESTC1	Baseline	Water	Slurry	*Δw*	*Δs*	*Δs-Δw*
T1	6.738 ± 0.181	5.745 ± 0.089	5.483 ± 0.156	0.993	1.255	0.262
T2	6.964 ± 0.134	6.495 ± 0.090	5.947 ± 0.329	0.469	1.017	0.548
T3	6.761 ± 0.077	6.363 ± 0.256	5.882 ± 0.217	0.399	0.879	0.481
T4	6.129 ± 0.121	5.012 ± 0.172	4.830 ± 0.186	1.117	1.300	0.182
T5	6.559 ± 0.090	5.259 ± 0.180	4.892 ± 0.232	1.300	1.667	0.367
Avg.	6.630	5.775	5.407	0.856	1.224	0.368

Effective setting 2 (ESTC2) included five trials. For each trial, water and slurry shared the same baseline. ESTC2 explored a 10-minute immersion length. STR average

(mean) $\log_{10}$ cfu/mL values and respective standard deviations recovered from the stomached wing rinse are reported in Table 4.3.3. For all trials, slurry provided higher pathogen reduction than water, averaging 0.261 $\log_{10}$ more between all five trials. In the best trial, T4, slurry yielded 0.549 $\log_{10}$ added reduction over chilled water.

Table 4.3.3 Effective Setting 2: Slurry Dosatron 1:22, 50 ppm PAA, for 10 minutes of immersion chilling

	Salmonella Typhimurium $\log_{10}$ cfu/mL					
ESTC2	Baseline	Water	Slurry	*Δw*	*Δs*	*Δs-Δw*
T1	6.041 ± 0.301	5.088 ± 0.404	4.761 ± 0.210	0.953	1.280	0.327
T2	6.373 ± 0.130	4.883 ± 0.267	4.772 ± 0.147	1.490	1.601	0.111
T3	6.725 ± 0.124	5.060 ± 0.165	4.998 ± 0.172	1.665	1.727	0.062
T4	6.029 ± 0.148	5.724 ± 0.272	5.175 ± 0.153	0.305	0.854	0.549
T5	5.828 ± 0.235	5.102 ± 0.187	4.844 ± 0.296	0.727	0.985	0.258
Avg.	6.199	5.171	4.910	1.028	1.289	0.261

Effective setting 3 (ESTC3) included three trials. ESTC3 explored 10 minutes immersion length. ES3 slurry settings kept a constant Dosatron 1:30 and "effective" 50 ppm initial PAA dependent upon slurry ice fraction. STR average (mean) $\log_{10}$ cfu/mL values and respective standard deviations recovered from the stomached wing rinse are reported in Table 4.3.4. In two trials, slurry provided slightly higher STR reductions, averaging 0.068 $\log_{10}$ more than chilled water. In the best trial, T1, slurry yielded 0.149 $\log_{10}$ added reduction over chilled water. T3 had no differences between the media: the 10-minute immersion times may not have been long enough for added pathogen reductions at the factor settings.

Table 4.3.4 Effective Setting 3: slurry Dosatron 1:30, 50 ppm PAA, for 10 minutes of immersion chilling

	Salmonella Typhimurium $\log_{10}$ cfu/mL					
ESTC3	Baseline	Water	Slurry	*Δw*	*Δs*	*Δs-Δw*
T1	5.669 ± 0.087	4.379 ± 0.254	4.230 ± 0.207	1.290	1.438	0.149
T2	5.589 ± 0.156	4.442 ± 0.174	4.335 ± 0.301	1.147	1.254	0.107
T3	7.214 ± 0.065	5.996 ± 0.111	6.047 ± 0.122	1.218	1.167	-0.051
Avg.	6.157	4.939	4.871	1.218	1.286	0.068

Effective setting 4 (ESTC4) included three trials. For each trial, water and slurry share the same baseline. ESTC4 explored 20 minutes immersion length. Ice slurry kept a constant setting of Dosatron 1:30 and "effective" 50 ppm PAA dependent upon ice fraction. Chilled water kept a constant setting of 50 ppm PAA. STR average (mean) $\log_{10}$ cfu/mL values and respective standard deviations recovered from the stomached wing rinse are reported in Table 4.3.5. For all trials, slurry provided higher reductions over water, averaging 0.279 $\log_{10}$ more. In the best trial, T2, slurry yielded 0.357 $\log_{10}$ added reduction over chilled water.

Table 4.3.5 Effective Setting 4: Slurry Dosatron 1:30, 50 ppm PAA, for 20 minutes of immersion chilling

	Salmonella Typhimurium $\log_{10}$ cfu/mL					
ESTC4	Baseline	Water	Slurry	*Δw*	*Δs*	*Δs-Δw*
T1	6.395 ± 0.129	5.011 ± 0.115	4.848 ± 0.126	1.384	1.547	0.163
T2	6.854 ± 0.160	5.365 ± 0.369	5.008 ± 0.220	1.489	1.846	0.357
T3	6.683 ± 0.185	5.483 ± 0.176	5.166 ± 0.174	1.200	1.517	0.317
Avg.	6.644	5.286	5.007	1.358	1.637	0.279

Table 4.3.6 details the results of combining all trial groups into a single sample representative per effective setting treatment combination by group with associated p-

values. Three of the four effective setting treatment combinations reductions between slurry and water were statistically significant ($P \leq 0.05$) when combining all groups' representatives into a single sample set. ESTC3 reductions were similar between slurry and water in all three trials. Given 10-minute immersion lengths at Dosatron 1:30, antimicrobial exposure time might not long enough to see differences between the two chilling media.

Table 4.3.6 Effective Setting Treatment Combinations for Wing Immersion Experiments

Effective Setting Treatment Combinations for Wing Immersion Chilling						
ESTC#	Δ_W	Δ_W $P=$	Δ_S	Δ_S $P=$	$\Delta_S - \Delta_W$	Δ_S-Δ_W $P=$
ESTC1	0.850	<.0001*	1.222	<.0001*	0.372	0.017*
ESTC2	1.069	<.0001*	1.325	<.0001*	0.255	0.400
ESTC3	1.238	<.0001*	1.264	<.0001*	0.026	1.000
ESTC4	1.358	<.0001*	1.637	<.0001*	0.279	0.595

As mentioned in Chapter 3, the agitation power (i.e., air supply with heat of compression effects) warmed the media faster than seen in the WOGs investigation. With the addition of the air agitation displacement plates, the ice particles were still present at the end of 20-minute immersion lengths, though at less than 50% of the starting amounts. Yet even with the final slurry media increasing in the liquid portion, the results showed ice slurry's ability to increase pathogen reductions (presumably due to scrub during the initial phase of the experiment wherein solid ice was existent). Even when adjusting the PAA and salinity levels to have same effective initial concentrations, ice slurry provided

higher pathogen reductions over chilled water. These results further support the abrasion hypothesis and ice slurry as a viable alternative chilling medium.

Future tests need to include recording the weights of all wings and/or only using wings in an acceptable weight range. Many of the wings received for these tests had extra skin attached at the carcass separation site. For more consistent STR recovery, the excess skin needs to be removed before STR dip. A simple Dosatron adjustment calculation was used in this study. The calculation did not account for the brine solution density and may have contributed to chilled water tests undesirably having higher salinities than the initial chilled water. Even if the equation was corrected for brine density, the twist knob for Dosatron ratio change is not very accurate or precise. Later testing found that the predicted salinity, from the Dosatron ratio and calculated brine density, did not match the actual salinity in the produced media. Before further testing could be conducted to refine a new equation, Highland Refrigeration has to end the loan of the ice slurry machine.

CHAPTER 5. INITIAL INVESTIGATION OF WHOLE CARCASS SALT-UPTAKE TENDENCIES DURING IMMERSION CHILLING

5.1 Introduction

Salt content of the final meat products can also have regulatory concerns. Consumers are also increasingly aware regarding reduced sodium intake to prevent hypertension and cardio-related diseases (Broadway, 2011). Salt presence, dependent on concentrations, can accordingly result in labeling requirements and consumer avoidance.

Ice slurry utilizes a brine solution, made in the presently reported study with agricultural sodium chloride feed salt, to keep the slurry in a homogenous state (i.e., reduce ice agglomeration and media separation). Evoking the basic osmosis and diffusive principles, the saline ice slurry media may increase water and salt uptake of the whole carcasses.

A study was thus conducted to assess the effects of three chilling methods (saline chilled water, chilled water, and air chilling) on poultry carcass weight change, temperature change, and chloride concentrations after 60 minutes of chilling.

Sodium chloride has long been used for meat perseveration, flavor enhancement, and microbial prevention (Broadway, 2011). While consumers are concerned over sodium intake, NaCl is not the only form of sodium applied in processing; nitrate, nitrite, and ascorbate are types of sodium often added (Parpia et al., 2018).

5.2 Methods and Materials

To the author's knowledge, research regarding salt concentrations in poultry skin and meat has not been conducted. To address the lack of literature and methodology for salt recovery from meat product, an organic dry-ash method was adapted from which the total chloride concentration for each sample was determined using an ion chromatograph (IC).

5.2.1 Part I. UGA WOG chilling

WOGs were collected directly off a local commercial supplier's the post-evisceration line prior to final inspection that were feeding into the pre-chillers. WOGs were placed into plastic bags and added to transport bins and taken to the University of Georgia (UGA) where they were held until testing. Three treatments were examined on a given test day. Each treatment contained 5 WOGs per replicate. The three treatments were:

1. Air chill (in the refrigerator)
2. Chilled water in 50 ppm PAA
4. Chilled water, 50 ppm PAA, 4.5% salinity

5.2.1.1 Pre-Chill

WOGs were labeled using numbered leg tags and drained of surface liquids. Each WOG's initial weight was recorded, and breast meat temperatures were recorded.

5.2.1.2 Sixty Minute Immersion Chilling

For the dry air chill treatment, WOGs were placed breast-side up on racks within the walk-in refrigerator at 4^0C. This allowed air flow on all sides of the WOG. Treatments in chilled water were added to the chiller apparatus composed of a 44-gallon waste-container. Regarding chilled water supply, multiple 44-gallon trash bins were filled with ice water and stored in the freezer until the beginning of immersion chilling experiments. Prior to chilling, all the ice was removed, and the chilled water used in conjunction with connected airlines for agitation. PAA ppm and water temperatures were confirmed at times 0 minutes, 30 minutes, and 60 minutes.

5.2.1.3 Post-Chill

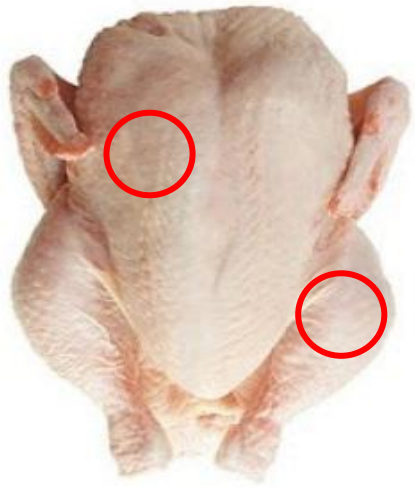

Figure 5.2.1 Meat sampling locations upon breast skin removal. White meat was collected from breast and dark meat collected from leg.

After chilling, all WOGs were drained to eliminate weight of any liquids. WOGs were again weighed and inner breast meat temperature was recorded. Three locations were chosen for cored-sampling sites (Figure 5.2.1) to provide one: breast skin sample,

white meat sample, and one dark meat sample per WOG. All samples (three locations per WOG) were weighed and stored on ice for transport. Skin samples were stored on a weigh dish and stored in a 3*3 bag (McMaster-Carr, Atlanta, GA) while meat samples were stored in 50 mL centrifuge tubes (VWR, Randor, PA).

5.2.2 Part II. GTRI sample analysis

The day of analysis, all samples were removed from the freezer and thawed to room temperature. Taking only the top 1.0 cm of meat for analysis, each sample was minced into smaller pieces. Each sample was finely minced for more uniform ashing. Each sample (minced) had, as close to ~1.0 g weighed out, and added to a sterile porcelain crucible. Crucibles were added to a muffle furnace for 18 hours. The furnace was heated to 600 ◦C at a ramp rate of 5°C/minute (Kalra, 1998). Crucibles were cooled to room temperature in the turned-off furnace overnight. All ashed samples were suspended in 10 mL deionized water. The entirety of each suspended ash crucible contents was added into 50 mL centrifuge tubes.

Capped IC tubes, containing 1 mL of dry-ashed and 4 mL deionized water, were analyzed on a Dionex ICS-3000 through an AS-18 column. Each sample ran for 30 minutes. Chloride concentrations were calculated using a standard calibration standard and curve.

5.2.2.1 Statistical Analysis

Chloride concentrations collected from the Dionex IC were corrected by combining sample starting weight and dilution factors. Chloride concentrations were presented per

sample mass as ppm/g. One-way ANOVA tests compared chloride concentrations of the 3 * 3 factorial (3 chilling treatment types x 3 sampling locations) presented with mean ppm/g ± SD. Tukey post-hoc analysis was used to determine differences between replicate means ($P \leq 5\%$).

5.3 Results and Discussion

This initial investigation included three replicates (45 carcasses; total 135 samples). Chloride was recovered from the skin and meat samples using a standard organic dry-ash method. The total salt concentration for each sample was determined using an ion chromatograph (IC). Statistical analysis was performed from each sample's corrected IC data (combining sample weight and dilution factors). Chloride concentration was calculated as ppm per gram of sample. One-way ANOVA tests were used to compare chloride concentrations (ppm/g) given groups defined by chilling treatment and carcass skin/meat sampling type considering mean ± SD (p-value = 0.05). (Table 5.3.1).

Initial results show that, of all nine groups (i.e., 3 chilling types * 3 sampling types), Initial results show that, of all nine groups (i.e., 3 chilling types * 3 sampling types), 4.5% salinity chilled water skin samples were the only statistically significant group ($p = <0.0001$) in chloride ppm/g (Table 5.3.2). The 4.5% salinity skin samples (38.1 ppm/g) averaged 14x and 22x higher than chilled water and air chilled skin samples, respectively. Results show no statistical significance ($p > 0.05$) between white and dark meat samples between all three chilling forms.

Of the three chilling methods, two served as controls. Air chilling served as a no immersion treatment control. In air chilling methods no immersion water was absorbed

into the skin and none of the carcasses' naturally occurring salt was released into the chilling water. Chilled water served as a water immersion control. The chilled water group allowed for comparison against the 4.5% salinity chilled water carcasses' differences in salt.

Initial and final temperatures and weights, respectively, were compared between the three chilling methods (Table 5.3.3 and Table 5.3.4). Initial temperature (T_i) and carcass mass (M_I) were compared to final temperature (T_f) and carcass mass (M_f) to later determine if a relationship between salinity, chilling length, and mass change could be determined.

Table 5.3.1 Average chloride recovered, ppm/g, from all nine groups.

Chilling method by sample location	Chloride ppm/g	
	Mean	SD
4.5% Chilled Water: Thigh	5.674	1.677
4.5% Chilled Water: Skin	38.077	32.576
4.5% Chilled Water: Breast	2.352	0.603
Air Chilled- Thigh	2.687	0.666
Air Chilled - Skin	2.675	0.543
Air Chilled - Breast	2.121	0.611
Chilled Water: Thigh	3.572	0.480
Chilled Water: Skin	1.681	0.761
Chilled Water: Breast	3.143	0.550

Table 5.3.2 Tukey Post-Hoc **d**ifferences report showcasing 4.5% salinity chilled water skin samples differences in average mean ppm/g to all other eight groups.

Ordered Differences Report			
Level	- Level	Chloride ppm/g Difference	p-Value
4.5% Chilled Water: Skin	Chilled Water: Skin	36.397	<.0001*
4.5% Chilled Water: Skin	Air Chilled - Breast	35.956	<.0001*
4.5% Chilled Water: Skin	4.5% Chilled Water: Breast	35.725	<.0001*
4.5% Chilled Water: Skin	Air Chilled - Breast	35.402	<.0001*
4.5% Chilled Water: Skin	Air Chilled- Thigh	35.390	<.0001*
4.5% Chilled Water: Skin	Chilled Water: Breast	34.934	<.0001*
4.5% Chilled Water: Skin	Chilled Water: Thigh	34.505	<.0001*
4.5% Chilled Water: Skin	4.5% Chilled Water: Thigh	32.404	<.0001*

Table 5.3.3 Carcass internal breast meat temperature reduction after 60 minutes of chilling.

Chilling Method	T_i (°C)	T_f (°C)	ΔT (°C)
Air Chill	30.167 ± 1.160	17.200 ± 1.612	12.967 ± 0.743
Chilled Water	30.400 ± 1.183	9.800 ± 0.775	20.600 ± 1.121
4.5% Salinity	30.200 ± 0.775	10.067 ± 0.258	20.133 ± 0.743

Table 5.3.4 Carcass weight change after 60 minutes of chilling.

Chilling Method	M_I (grams)	M_f (grams)	ΔM (grams)
Air Chill	1408.280 ± 230.385	1391.96 ± 226.457	-16.320 ± 5.677
Chilled Water	1424.050 ± 162.353	1463.640 ± 175.066	42.588 ± 23.993
4.5% Salinity	1482.150 ± 210.198	1516.750 ± 212.270	34.593 ± 11.991

The dry air chilling method cooled the internal breast meat slower than either immersion chilling methods (i.e., 12.67°C reduction compared to 20.60°C and 20.13°C). Chilled water carcasses had the largest weight gain (42.59 g) while air chilled carcasses lost weight (-16 g) during 60 minutes of chilling.

Our results address salt-uptake concerns when ice slurry medium is used for immersion chilling. Initial findings indicate that salt concentrations increase in the skin and do not affect white or dark meat. The skin acts as a barrier that hinders salt penetration into the white and dark meat. If the salt is found present in the skin, plants manufacturing skinless parts could more readily utilize ice slurry technology. Further testing will examine higher salinity immersion chilling to see if salt can penetrate through skin at extreme slurry liquid phase salinities. Testing will also be conducted on damaged (skin cuts or lesions) WOGs to observe salt concentrations in the same three sampling locations.

Chloride accounts for 60% of sodium chloride salt, and the other 40% is sodium (NAMI, 2015). Further testing is needed to confirm if the sodium and chloride are found in the same locations and calculated concentrations. The consumer concern regards sodium content, and it must be confirmed or corrected whether what is occurring with the chloride is mirrored by the sodium. If the sodium transport proportionately matches that of the chloride, these initial results allow sodium determination. The alternative is that the sodium could be acting and penetrating in a different manner.

CHAPTER 6. CONCLUSION

Approximately 95% of all U.S. processing plants use immersion chilling to cool carcasses to hinder pathogen growth and presence. This thesis focused upon poultry chilling, principally to determine ice slurry's validity as an immersion chilling medium. The ice slurry was hypothesized to provide an abrasive quality that erodes and dislodges skin-attached pathogens for enhanced bacterial reductions during immersion chilling. Traditional chilled water-ice mixtures used in immersion systems do not provide the thermal and scrubbing benefits of ice slurry.

Rowe's thesis (Rowe, 2016) reports data for ice slurry chiller medium feasibility, electrical energy demand, and thermal cooling capacity. The approach of ice slurry chilling medium was supported with Rowe's extensive findings.

This thesis addresses ice slurry as a response to food safety issues that the poultry industry faces: *Salmonella* contamination on WOGs and wings. To address food-borne Salmonellosis concerns, a strain of traceable *Salmonella* was obtained from the USDA. By using an antibiotic nalidixic acid resistant *Salmonella* Typhimurium, all media were treated with 100 ppm nalidixic acid to rid other prevalent bacterial types. The STR was then added to a buffered peptone solution, a known pre-enrichment media for *Salmonella*, for injured cell recovery (Gamble, 2016). We combined two nutrient rich media to avoid reporting false negative STR results. The effects of the recovery BPW media with non-specific aerobic plates were thought to provide ample nutrients for all injured STR cells' recovery.

The bacterial load level inoculated onto the WOGs and wings (10^6 CFU/mL concentration) allowed ample spreading and attaching of the bacterium during the heating phase of all studies. Heating utilized a hot water-bath, where the individually bagged samples were heated to 35°C -37°C to allow bacteria to thrive at their optimum growth temperature.

The heating phase allowed bacterial new environmental acclimation to the skin and may also have resulted in an increase in STR replication and thus concentrations. The increase concentrations affected baseline-group recovered concentrations between trials and experiments. The variation between baseline concentrations posed statistical concerns. When treatment combination replicates/trials were combined into a single sample set, the result was a large standard deviation and higher p-values than seen in replicate by replicate comparisons.

All experiments contained a comparative control group deemed the "baseline". Baseline groups provided representative post-heating/pre-chilling STR concentrations to allow determination of bacterial reductions post-chilling of either slurry or water. Baseline and corresponding chiller groups allowed determination of pathogen reduction where the reduction was attributed to the medium type and its treatment combinations. Using JMP statistics software, all treatment combinations were analyzed using one-way ANOVA tests and compared by:

1. Treatment combination replicates for mean, standard deviation, and Tukey post-hoc differences and p-value significances

a. Chapter 2 WOG experiments (TC 2-7) contained four groups in a treatment combination; ice slurry, ice slurry baseline, chilled water, and chilled water baseline
b. Chapter 3 and Chapter 4 wing chilling experiments (MCTC 1-4 and ESTC 1-4) contained three groups in a treatment combination; shared baseline, ice slurry, and chilled water

2. Combined replicates from each treatment combination into one sample set

Findings presented a primary trend of ice slurry capacity for increased pathogen reduction over chilled water. TC3, TC7, ESTC1 averaged 0.313, 0.316, 0.372 $\log_{10}$ higher reduction at P=0.04, P=0.015, and P= 0.017 respectively. Some of the treatment combinations showed higher STR reductions due to chilled water. In these trials where chilled water outperformed slurry's antimicrobial effect, we observed that the slurry was not preserved in a homogenous mixture and the test subjects were not sufficiently agitated. In these cases, it could not be concluded that chilled water was the better pathogen reducing medium, but more explicitly these cases formed a confirmation of the necessary components of adequate salinity levels and agitation in keeping a well-mixed slurry.

Wing and WOG experiments reflected each other in STR reductions trends in the average enhanced STR reduction due to ice slurry. Not only can ice slurry be used in the primary chiller, but the results support is application in any immersion chilling pre- or post- applications. From all of the treatment combinations, specifically for PAA, 20 ppm and 80 ppm did not deliver the hypothesized effects. At the low concentration, 20 ppm may not have been a strong enough antimicrobial for the 20-minute immersion times,

while the highest concentration applied, 80 ppm, did not yield an increase in pathogen reduction. At 80 ppm, the heightened PAA level may have decayed faster due to the saline composition of the ice slurry. The target concentration is 50 ppm PAA for the pathogen reduction efficacy from the ice slurry's abrasion and antimicrobial activity.

All poultry products used in STR-pathogen experiments were donated from a local processor as a post-chiller meats. In all experiments, there were uncontrollable extraneous variables thus dependent upon the processing plants antimicrobial application (chemical types and concentrations applied) and chilling dwell length. Between experiments the carcasses and wings varied in size, color, fat composition, water drainage during heating, and excess skin attachments.

The experimental immersion medium (slurry or water) composition feeding from the ice slurry generator was dependent on the pre-made brine and City of Atlanta water. It was observed that water temperatures varied seasonally, during warmer months it was more difficult to generated thicker slurry. Seasonal temperature variability showed that even when using the same Dosatron ratio between tests, the ice fraction may vary. The project team adapted to slurry thickness by recording the ice fractions between tests. Additionally, the water properties (pH, hardness, microbe presence, and mineral content) were not tested. Water properties may have impacted STR reductions. In future ice slurry immersion studies, water properties need to be measured and possibly controlled for.

Meat flavor and appearance are important qualities that are easily affected by processing additives. While it is known how antimicrobial additives affect meat quality, there is not ample literature on salt's impact. The project has reported on ice slurry's thermal and microbial benefits, and has now investigated the effects on the poultry meat

product with salt additives. The brine solution for all ice slurry test was made using and agricultural feed salt, the salt was mainly composed of sodium chloride. Sodium is added in many forms during processing so to ensure any meat flavor changes were due to the ice slurry salt-brine, chloride was the dependent factor analyzed for concentration changes.

Salt-uptake studies were conducted to track the meat and skin changes in chloride concentration. All WOGs used in these studies had three sample types collected: breast skin and top 1 cm of white-breast and dark-thigh meat. From these locations, we were able to determine the chloride concentrations (ppm/g) of each sample (total 135 samples). Only the top 1 cm of meat was analyzed as that is where more of the salt would penetrate. Taking meat samples from deeper depths would give a diluted concentration of the top layer. Our results show carcasses natural yield 2.0-3.0 ppm/g chloride in skin, white, and dark meats prior to chilling.

The carcass skin samples from 4.5% saline chilled water increased to 38.01 ppm/g after 60 minutes of chilling. This was 14.23x and 22.63x higher than the air chilled skin and chilled water skin samples respectively. These salt studies investigate slurry's salt-brine impact on WOGs during immersion chilling. Additionally it demonstrates salt uptake tendencies of plants generating "enhanced meat products" by margination or saline chilled water immersion methods.

Future tests for salt-uptake studies will include a 12% salinity chilled water concentration. The industry maximum used saline level would conservatively be 12% in chilled water based upon feed water's 3-4% salinity and a lofty 2/3 ice fraction attainment. Additionally, the labeling requirements will be addressed later for these

studies. Next steps for the salt-uptake tests will define a relationship between carcass weight change (g) and skin salt-uptake.

Ice slurry provides faster carcass cooling times, increased pathogen reduction, and possibly more amenable energy and water costs. Off-peak hour generation of the ice slurry that may later be pumped is a more financially attractive option than peak-hours cooling demand. The faster chilling times and higher heat sink abilities of ice slurry can reduce the amount of chilling medium (hence water) needed each day. Ice slurry's colder temperatures, compared to chilled water, and its hypothesized abrasive quality provide better pathogen reductions. This multi-year study of ice slurry further supports the use of ice slurry in poultry immersion chillers. Ice slurry is a more sustainable approach for water and energy consumption for the poultry industry.

CHAPTER 7. REFERENCES

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